Host JAK/Stat activity is a target of endoparasitoid wasp virulence strategies

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Abstract

Innate immune responses depend on the action of multiple conserved signaling pathways. Pathways important for activation of humoral immune responses following microbial infection are well-characterized in the genetic model species *Drosophila melanogaster*, but our understanding of fly cellular immunity, and how parasites suppress it, is relatively fragmentary. Fly larvae mount a coordinated cellular immune response following infection by endoparasitoid wasps, characterized by the production of specialized blood cells called lamellocytes that form a tight capsule around wasp eggs in their hemolymph. The conserved JAK/Stat signaling pathway has been implicated in lamellocyte proliferation and is required for successful encapsulation of wasp eggs. Here we show that activity of Stat92E, the *D. melanogaster* Stat ortholog, is induced in the fat body and hemocytes following wasp infection. Virulent wasp species are able to suppress activation of a Stat92E activity reporter during infection, suggesting they target upstream activation of this pathway as part of their virulence strategies. Furthermore, two wasp species (*Leptopilina guineaensis* and *Ganaspis xanthopoda*) suppress phenotypes associated with gain-of-function mutations in *hopscotch*, the *D. melanogaster* JAK ortholog, showing that they inhibit JAK/Stat activity downstream of JAK. Our data demonstrate that endoparasitoid wasp virulence factors block JAK/Stat signaling to overcome fly immune defenses.

Introduction

Innate immune mechanisms are evolutionarily ancient defenses against pathogens and many immune signaling pathways, such as the Toll/Nf-κB pathway, are conserved from insects to mammals. In the fruit fly *D. melanogaster*, the closely related Toll and Imd pathways are responsible for the humoral immune response against microbial infections, culminating in the release of antimicrobial peptides into the blood to eliminate these pathogens (Lemaitre et al., 1995, 1996). Similarly, Toll-like receptors in humans recognize specific pathogens and signal through NF-κB to produce antimicrobial peptides, cytokines, and interferons (Valanne et al., 2011). Parallels between the humoral immune mechanisms of flies and humans suggest that study of cellular immune mechanisms in flies may also lead to a broader understanding of human immunity. In addition to the humoral immune response to infection, the *Drosophila* immune system can mount a cellular response to eliminate pathogens by encapsulation. It has become increasingly apparent that the mechanisms that control this cellular response and host-pathogen recognition rely on conserved proteins that are also important for human immunity (Howell et al., 2012; Mortimer, 2013; Mortimer et al., 2012).

Fly larvae constitutively produce two circulating hemocyte (blood cell) types, plasmatocytes and crystal cells. Plasmatocytes make up almost 95% of the hemocytes in circulation and are responsible for phagocytosis of microbes, while crystal cells store a pro-enzyme required to generate melanin during wound healing (Lanot et al., 2001). A third hemocyte type, the lamellocyte, is produced specifically to encapsulate pathogens

too large to be phagocytosed, such as endoparasitoid wasp eggs. In this encapsulation response, plasmatocytes are the first hemocytes to bind to the foreign tissue, and they signal pro-hemocytes in the lymph gland (the larval hematopoietic organ) as well as other circulating plasmatocytes (via the intermediate podocyte form) to differentiate into lamellocytes (Anderl et al., 2016; Honti et al., 2010). The lamellocytes then migrate towards, adhere to, and spread over the foreign tissue, killing the encapsulated object (Carton et al., 1986; Russo et al., 1996; Schlenke et al., 2007).

Numerous wasp species infect fruit flies in nature. These wasps inject their eggs along with venom into the hemocoel of fly larvae and pupae. If the eggs are not killed, they will hatch into wasp larvae, which begin to eat the flies from the inside out, eventually killing the fly host and eclosing as adult wasps. *Leptopilina heterotoma* is a *D. melanogaster* parasite that suppresses cellular immunity using a venom factor(s) that specifically lyses host lamellocytes (Chiu and Govind, 2002; Rizki and Rizki, 1990, 1992; Rizki et al., 1990; Schlenke et al., 2007), suggesting that inhibition of the production or action of lamellocytes may be an effective parasite virulence strategy. Dominant, gain-of-function mutations in the *D. melanogaster* JAK ortholog, *hopscotch* (*hop*), cause excess hemocyte production and differentiation of lamellocytes in uninfected flies, and loss-of-function mutants have reduced hemocyte counts and weakened encapsulation ability (Luo et al., 1995; Sorrentino et al., 2004; Zettervall et al., 2004). Thus, wasp parasites might suppress lamellocyte production and encapsulation by blocking regulators, signaling components, and downstream targets of JAK/Stat activity. In this study, we show that a panel of virulent endoparasitoid wasp species can suppress JAK/Stat activity upstream of Stat or downstream of JAK. Our findings demonstrate that parasite virulence mechanisms have converged on a strategy of host JAK/Stat activity disruption.

Results

To investigate the tissue specific role of JAK/Stat activity in the production of lamellocytes, we expressed a constitutively active form of Stat92E (Stat92E^{CA}) in these immune tissues (Ekas et al., 2010). Expression of Stat92E^{CA} by the hemocyte-specific driver *He-Gal4* induced differentiation of lamellocytes. While podocyte and plasmatocyte numbers did not significantly change following expression of Stat92E^{CA} in blood cells, the trend of decreased plasmatocytes and increased podocytes suggests that lamellocytes differentiated from plasmatocytes via a podocyte intermediate as has been shown previously (Stofanko et al., 2010). Expression of Stat92E^{CA} in the fat body (the major site for humoral immune signaling) using the fat body-specific driver *C833-Gal4* promoted lamellocyte differentiation without significant changes to other hemocyte populations. Expression of Stat92E^{CA} specifically in the Posterior Signaling Center (PSC) of the lymph gland, the main hematopoietic organ of *D. melanogaster* using *Dot-Gal4* did not induce constitutive lamellocyte differentiation (Figure 1, Table S1).

Previous studies using *Stat92E* loss-of-function mutants (Sorrentino et al., 2004) have shown that JAK/Stat signaling is necessary for the *D. melanogaster* encapsulation response. However, these studies have only investigated the effect of global JAK/Stat signaling on the immune response. To characterize the tissue specificity of JAK/Stat activity in the context of the immune response to wasp infection, we challenged Stat92E

activity reporter flies (*10XStat92E-GFP*)(Bach et al., 2007) with a panel of diverse wasp species (Figure S1). These flies express GFP in response to JAK/Stat pathway activation such that GFP fluorescence can be used as a measure of pathway activity. In uninfected reporter flies, Stat92E activity is observed in cells of the gut and the brain (Figure S2), and no expression is observed in immune tissues (hemocytes, fat body, and lymph gland) (Figure 2). Previous reports (Márkus et al., 2005) have shown that sterile wounding is sufficient for lamellocyte production, so we used a sterile wound as a positive control for the induction of an immune response. While lamellocytes were observed following wounding, no Stat92E activity was observed up to 48 hours after piercing. However, challenge with the avirulent wasp, *Leptopilina clavipes* (strain LcNet), a wasp that induces a healthy immune response and is usually encapsulated by *D. melanogaster,* altered the pattern of Stat92E activity: at 48 hours following LcNet infection, GFP fluorescence was detected in hemocytes and fat body (Figure 2).

Immune challenge by a diverse panel of wasp species induced wasp-specific patterns of Stat92E activity (Figure 2; Table 1; Figure S3). In comparison to *L. clavipes*, other, more virulent wasps induced lower levels of GFP fluorescence in hemocytes 24 hours (Figure 3A) and 48 hours (Figure 3B) after wasp infection. A similar pattern was observed with respect to Stat92E activity in the fat body, with the exception of the *Leptopilina guineaensis* strain LgCam, which showed the highest level of Stat92E activity in the fat body following infection. Interestingly, the closely related *L. guineaensis* strain LgSA did not induce Stat92E activity in the fat body (Table 1).

Our results show that JAK/Stat signaling is active in hemocytes and the fat body following wasp infection but can be modulated by wasp virulence mechanisms. Next, we wanted to test whether wasps can target lamellocyte production downstream of JAK/Stat activation. To do this, we used the *hop* gain of function mutant fly strain, $hop^{Tum\text{-}l}$ (Hanratty and Dearolf, 1993; Luo et al., 1995). At high temperatures, these flies produce constitutively high numbers of lamellocytes. These adhesive cells have no target for encapsulation and often bind to each other, resulting in melanized cell nodules that are referred to as "melanotic tumors." We infected $hop^{Tum\text{-}l}$ flies with a panel of wasps and observed the effect of wasp infection on penetrance of the tumor phenotype. Two wasp species, *L. guineaensis* (strain LgCam) and *Ganaspis xanthropoda* (strain GxHaw), were able to significantly reduce the penetrance of the tumor phenotype (Figure 4). While not significant, a similar trend of lower tumor penetrance was observed for closely related strains LgSA and GxUg.

We measured hemocyte numbers at 24 and 48 hours following wasp infection to determine how wasp virulence strategies affect hemocyte production in $hop^{Tum\text{-}l}$ larvae (Figure 5, Table 2). In wild type flies, by 24 hours after infection plasmatocytes have bound to the wasp egg and lamellocytes have entered circulation, beginning the process of encapsulation. By 48 hours, lamellocytes are incorporated into the capsule, and melanization begins (Mortimer et al., 2012). At 24 and 48 hours post-infection, the avirulent wasp LcNet induces higher numbers of circulating lamellocytes even in the $hop^{Tum\text{-}l}$ background, indicative of a successful immune response (Table 2). *L. boulardi* is a specialist on *D. melanogaster* and evades encapsulation by attaching to the fly's own

tissue (Rizki et al., 1990). In the *hop*$^{Tum-l}$ background, *L. boulardi* (strain Lb17)-infected larvae mount an immune response and increase the number of lamellocytes in circulation at 48 hours post-infection, much like LcNet (Table 2B). Interestingly, several wasp strains (LhSw, LvPhil, LgCam, and LgSA) all decreased relative circulating podocyte numbers 24 hours after infection (Table 2A).

Specifically, to look more closely at the possible mechanisms of wasp virulence in the wasp species that significantly reduced tumor penetrance (*L. guineaensis* and *G. xanthopoda*), we focused on the ability of these species to affect hemocyte identity and number. At 24 hours after wasp infection, LgCam-infected flies produced a significantly higher percentage of circulating plasmatocytes and lower percentage of podocytes than controls (Figure 5A, Table 2A). At 48 hours post-infection, LgCam-infected larvae had lower numbers of circulating lamellocytes. A closely related wasp, LgSA, also decreased the percentage of circulating podocytes and increased relative plasmatocytes at 24 hours after wasp infection (Figure 5A, Table 2A). While GxHaw did not have a significant effect on hemocyte number, the closely related strain GxUg significantly reduced the percentage of circulating lamellocytes 48 hours after infection (Figure 5B, Table 2B).

To investigate the basis of effects on tumor penetrance, we hypothesized that there may be a correlation between tumor penetrance and relative hemocyte numbers. We find that there is a significant correlation between relative lamellocyte numbers to tumor phenotype penetrance 48 hours after infection, in which high tumor penetrance is associated with a higher percentage of circulating lamellocytes (Figure 6).

These data show that ectopic activation of Stat92E in hemocytes and fat body induces lamellocyte differentiation, and that Stat92E activity in these tissues is altered following wasp infection. The virulent wasp LgCam decreased tumor penetrance in the *hop*$^{Tum-l}$ mutant background, and Stat92E activity in hemocytes following attack (Figures 3,4), although it did induce high levels of Stat92E activity in the fat body, suggesting that LgCam may be specifically targeting Stat92E in hemocytes. To test this hypothesis, we expressed Stat92E^{CA} in hemocytes and measured the encapsulation rate of LgCam eggs. Interestingly, while none of the eggs in control larvae were encapsulated, flies expressing Stat92E^{CA} in hemocytes encapsulated LgCam 13% of the time (Figure 7).

Discussion

Here we have shown that Stat92E is active in hemocytes and the larval fat body following wasp infection to produce lamellocytes that are essential for the wasp encapsulation response. JAK/Stat signaling does not seem to be acting in the signaling cells of the lymph gland of the fly to produce the lamellocytes involved in the encapsulation response, as expression of Stat92E^{CA} in this region did not lead to an increase in circulating lamellocytes. Stat92E activity in hemocytes and fat body does induce lamellocyte differentiation, although in different ways. Hemocyte-specific Stat92E^{CA} expression leads to the production of lamellocytes. This is consistent with studies that have shown through lineage tracing experiments that plasmatocytes differentiate into lamellocytes in the context of the immune response against parasitic

wasps (Honti et al., 2010; Stofanko et al., 2010). Our data suggest that this differentiation may be stimulated by JAK/Stat activity in blood cells.

Interestingly, expression of Stat92E^{CA} in the fat body induced higher levels of circulating lamellocytes, suggesting that JAK/Stat activity in the fat body may act through an unknown, non-autonomous signaling mechanism to induce production of new hemocytes that differentiate into lamellocytes. The origin of this lamellocyte population is unclear from these data, but two hypotheses can be suggested. First, lamellocytes can be produced in the lymph gland from prohemocytes (Evans et al., 2003). Perhaps a cytokine signal downstream of Stat92E activation is secreted by the fat body to stimulate lamellocyte differentiation and release of new hemocytes into circulation from the lymph gland. A second hypothesis could be that a cytokine produced by JAK/Stat signaling in the fat body is sent to a population of hemocytes known as sessile hemocytes. These blood cells remain attached to the interior of the larval cuticle and remain out of circulation in wild type, uninfected flies. When marked with GFP, these cells can be observed as a distinct banding pattern on the fly cuticle. However, upon wasp infection, this banding pattern disappears, suggesting that the sessile hemocytes have entered circulation (Márkus et al., 2009; Zettervall et al., 2004). Differentiation of these cells into lamellocytes may be the source of excess lamellocytes we observe following expression of Stat92E^{CA} in both the fat body and hemocytes.

Consistent with the induction of lamellocyte differentiation by Stat92E^{CA} expression, we also show that wasp infection leads to Stat92E activity in the hemocytes and the fat body. Interestingly, more successful or virulent wasps seem to induce lower levels of Stat92E activity in their fly hosts than avirulent wasps (LcNet and LcAtl). This suggests that successful wasp parasites evade or suppress upstream *D. melanogaster* immune mechanisms, direct components of JAK/Stat signaling, or downstream feedback loops as a virulence strategy. The requirement for Stat92E activity in the encapsulation response (Sorrentino et al., 2004) supports the idea that the JAK/Stat pathway could be a likely target for wasp virulence factors.

Two virulent wasps, LgCam and GxHaw, not only blocked Stat92E activity in hemocytes, but also decreased the penetrance of the tumor phenotype in *hop*$^{Tum\text{-}l}$ flies. This suggests that LgCam and GxHaw virulence strategies either target JAK/Stat activity directly at the level of Stat92E activity or act both upstream and downstream of JAK/Stat activation. Here we investigated the relationship of tumor phenotype to hemocyte composition of infected larvae. Low tumor penetrance significantly correlates with low relative lamellocyte numbers at 48 hours post-infection (Figure 6). Specifically, LgCam, which significantly reduced the tumor phenotype of *hop*$^{Tum\text{-}l}$ flies, also significantly reduced the number of circulating lamellocytes 48 hours post-infection. These data support the hypothesis that wasps that inhibit tumor phenotype penetrance do so by blocking lamellocyte differentiation directly at the level of Stat92E activity.

To look more closely at the possible mechanisms of wasp virulence, we focused on the wasp strains that significantly reduced tumor phenotype penetrance (LgCam and GxHaw) and other strains of the same species that showed similar, though not significant trends

(LgSA and GxUg). *L. guineaensis* strains (LgCam and LgSA) caused significant increases in circulating host plasmatocytes and decreases in podocytes at 24 hours after wasp infection. It has been shown that lamellocytes differentiate from plasmatocytes via the intermediate podocyte state (Stofanko et al., 2010). Our data suggest that cells that might differentiate into lamellocytes during a regular immune response may not be activated due to *L. guineaensis* virulence. Following infection by the avirulent wasp LcNet, *D. melanogaster* mounts a successful immune response. In the $hop^{Tum\text{-}l}$ background, LcNet induces higher lamellocyte numbers, and flies infected by LcNet continue to increase lamellocyte production up to 48 hours after infection. In contrast, LgSA and GxHaw, wasps that showed a trend of low tumor penetrance, did not increase the lamellocyte population, and LgCam and GxUg decreased the number of lamellocytes at 48 hours. These data suggest that wasps that lower tumor penetrance may do so by inhibiting the number of lamellocytes in circulation. At 24 hours after infection GxHaw induced equivalent Stat92E activity in hemocytes in comparison to LcNet and LcAtl. This suggests that GxHaw may evade the immune response by altering the function of lamellocytes rather than their abundance via some other unknown mechanism. Similarly, LgCam induced high levels of Stat activity, primarily in the fat body (Table 1), but also lowered tumor penetrance. This suggests that LgCam may act directly at the level of Stat activity, preventing Stat-dependent transcription. To test this, we assayed the encapsulation ability of flies expressing $Stat92E^{CA}$ in hemocytes and found that LgCam eggs can be encapsulated in these flies, whereas no LgCam encapsulation was observed in control flies. These data suggest that LgCam may be blocking lamellocyte production by directly targeting Stat92E activity in hemocytes.

While we identified trends relating tumor phenotype penetrance to hemocyte numbers (Figure 6), it is also evident that individual wasps employ very specific virulence strategies against *D. melanogaster*. These mechanisms of interference should be investigated further to better understand the ways that natural parasites may manipulate conserved signaling pathways involved in host immune responses. *D. melanogaster* and its parasitic wasps is an excellent model system for studying these interactions. There are four JAKs and seven Stats in the human system. *D. melanogaster* has one JAK, (*hop*) and one Stat (*Stat92E*), a much simpler system with similar functionality and sequence conservation to the human JAK/Stat pathway (Agaisse and Perrimon, 2004). Since its discovery just over twenty years ago, the JAK/Stat pathway has been shown to play an essential role in mammalian hematopoiesis very similar to the mechanisms by which JAK/Stat signaling regulates cellular differentiation in *Drosophila*. For example, similar to the $hop^{Tum\text{-}l}$ model in *Drosophila*, mutations in JAK/Stat pathway components and regulators in humans have been associated with lymphomas and leukemias (Stark and Darnell, 2012). Much like the majority of human disease genes, the model genetic system *D. melanogaster* can be exploited to study the highly conserved JAK/Stat signaling pathway *in vivo*.

Materials and Methods

Insects

Flies were maintained on standard medium of cornmeal, yeast, and molasses. *He-Gal4*, *C833-Gal4*, *Dot-Gal4*, and $hop^{Tum\text{-}l}$ strains were obtained from the Bloomington Drosophila

Stock Center (Bloomington, IN). The Gal4-UAS binary system was used to express a constitutively active Stat92E trangene (*UAS-Stat92E*^{CA}) constructed in Erika Bach's lab (Ekas et al., 2010). The JAK/Stat reporter strain (*10xSTAT92E-GFP*) was constructed by Erika Bach (Bach et al., 2007).

Wasp strains (Figure S1) were obtained and maintained as described in (Kacsoh and Schlenke, 2012). Briefly, all wasps were maintained on the *D. melanogaster* strain Canton S, with the exception of *L. clavipes* (strains LcNet and LcAtl), which was maintained on *D. virilis*. Strain LbG486 was provided by D. Hultmark, LcNet was provided by J. van Alphen, and strain GxUg was provided by J. Pool. All other wasp strains were collected by the Schlenke lab.

Hemocyte Counts

All crosses and experiments were carried out at 25ºC with the exception of experiments carried out with *hop*$^{Tum-l}$ temperature sensitive mutants, which were incubated at 28ºC for up to 72 hours after a 24-hour wasp infection at 25ºC. For Stat92E^{CA} expression hemocyte counts, crosses between *Gal4* virgin females and *UAS-Stat92E*^{CA} or *w*^{1118} (wild type control) males were done at 25ºC, in three replicates per treatment. Wandering third instar larvae were selected for hemocyte counts. For *hop*$^{Tum-l}$ hemocyte counts, flies were allowed to lay eggs for 24 hours at 25ºC. Egg lay plates were then placed at 28ºC and incubated for 48 hours. Thirty early third instar larvae were picked from egg lay plates and transferred to 35mm diameter Petri dishes, in three replicates per treatment. For infection treatments, three female wasps were allowed to oviposit for 24 hours at 25ºC. Following wasp infection, wasps were removed and the infected and control (uninfected) larvae were allowed to incubate at 28ºC. Hemocytes were counted at 24 and 48 hours after being moved to 28ºC.

From each cross, three sets of 5 larvae were bled into 20 uL of 1X PBS with 0.01% PTU (to prevent blood cell lysis and melanization). The hemolymph dilution was transferred to a disposable hemocytometer (Incyto C-Chip DHC-N01). Hemocytes from sixteen 0.25 x 0.25 x 0.1 mm squares were counted for each replicate. Three hemocyte types were identified: plasmatocytes (small, dark cells), podocytes (larger, rounder cells with cytoplasmic projections), and lamellocytes (large, flat cells with cytoplasmic ring).

Imaging

Wasp infections of *10xSTAT92E-GFP* reporter flies were carried out as described above at 25ºC, and imaging took place 24 and 48 hours after wasp infection. Five larvae were dissected under immersion oil from each of three replicate plates. For quantification of *10xSTAT92E-GFP* corrected total cell fluorescence, 20 blood cells from each of the 5 larvae were analyzed for fluorescence in comparison to background using ImageJ software (Mortimer et al., 2013).

hop^{Tum-l} flies were imaged as pupae after 72 hours of incubation at 28ºC following wasp infection to allow adequate time for development of the tumor phenotype. Penetrance was determined by phenotyping larvae for presence or absence of melanotic tumors.

Statistics

Student's t-tests and linear regressions comparing penetrance and hemocyte data were conducted using GraphPad Prism 6.0 software.

Figure Legends

Figure 1. JAK/Stat activity in hemocytes and fat body is sufficient for lamellocyte production.
Lamellocyte numbers changed significantly when the constitutively active Stat92E^{CA} allele was expressed in hemocytes (*He-Gal4*) ($p<0.0005$) or fat body (*C833-Gal4*) ($p<0.005$), but not when expressed in the lymph gland (*Dot-Gal4*). Raw data are shown in Table S1.

Figure 2. Stat92E activity is induced in fat body and hemocytes following wasp infection. GFP expression in *10XStat92E-GFP* larval tissues 48 hours following infection by the avirulent wasp *L. clavipes* (LcNet; a-f) compared to age-matched uninfected larvae (g-l). Stat92E activity is induced in fat body (c,d) and hemocytes (e,f), but not in lymph gland (a,b), following wasp infection.

Figure 3. GFP activity in *10XStat92E-GFP* hemocytes following wasp infection by multiple wasp strains. GFP activity in *10XStat92E-GFP* hemocytes at (A) 24 and (B) 48 hours following wasp infection. Avirulent *L. clavipes* strains LcNet and LcAtl induce the highest Stat92E activity in comparison to multiple virulent wasp strains. Each data point represents the average corrected total cell fluorescence for 180 blood cells. Stat92E activity in hemocytes quantified by fluorescence and corrected for background in ImageJ. Significance versus control (*) and versus LcNet (▲) determined by student's t-test ($p<0.05$).

Figure 4. Wasp virulence strategies target host encapsulation ability downstream of JAK. Penetrance of *hop*$^{Tum-l}$ tumor phenotype 72 hours after wasp infection. Each data point represents the average penetrance of three replicates of thirty observed pupae. Significance determined by student's t-test versus daily uninfected control (* indicates $p<0.05$).

Figure 5. Hemocyte composition altered by wasps is associated with changes in *Hop*$^{Tum-l}$ tumor phenotype penetrance. Selected wasps alter plasmatocyte counts at 24 (A) and percent lamellocytes at 48 (B) hours after wasp attack. Each data point represents the average of three replicate hemocyte counts. Significance determined by student's t-test (* indicates $p<0.05$).

Figure 6. Correlations between relative hemocyte counts and tumor penetrance in wasp-infected flies. Relative (A) plasmatocyte and (B) podocyte counts 48 hours post-infection do not correlate with percent tumor penetrance. (C) In contrast, relative lamellocyte numbers are positively correlated with percent tumor penetrance.

Figure 7. *D. melanogaster* encapsulates LgCam when Stat92E^{CA} is expressed in hemocytes. LgCam encapsulation ability changed significantly when Stat92E^{CA} was expressed in hemocytes (*He-Gal4*) ($p<0.005$).

Table 1. ***10XStat92E-GFP*** **activity in fat body following wasp infection by multiple wasp strains.** (+) indicates estimated observed fluorescence intensity. Examples of qualitative levels of Stat92E activity are shown in Figure S3.

	24h post-infection	48h post-infection
Unattacked	None	None
Lb17	+	+
LbG486	++	++
Lh14	None	None
LhSw	None	None
LgCam	++++	++++
LgSA	None	None
LvPhil	None	+
LvHaw	None	+
LcNet	+++	+++
LcAtl	+++	+++
GxUg	None	None
GxHaw	None	None

Table 2. Wasp infection alters *hop*$^{Tum\text{-}l}$ hemocyte numbers. Hemocytes were counted at (A) 24 and (B) 48 hours post-infection of *hop*$^{Tum\text{-}l}$ larvae by the indicated wasp in three replicates. Red indicates a significant decrease in blood cell numbers/percent in comparison to average control; green indicates a significant increase in comparison to control; gray indicates no significant change compared to control (student's t-test, $p<0.05$).

A.

	CELL COUNTS				PERCENT of TOTAL CELLS		
24h post-infection	**Plasmatocytes±SEM**	**Podocytes±SEM**	**Lamellocytes±SEM**	**Total Cells±SEM**	**Plasmatocytes±SEM**	**Podocytes±SEM**	**Lamellocytes±SEM**
Control	121.3±14.1	274.3±50.0	27.3±4.1	423.0±58.5	39.9±11.8	64.0±3.4	7.0±2.1
Lb17	71.0±7.0	182.7±16.8	22.0±6.0	275.7±26.5	25.0±2.0	66.3±0.6	7.8±1.5
Control	121.3±14.1	274.3±50.0	27.3±4.1	423.0±58.5	39.9±11.8	64.0±3.4	7.0±2.1
LbG486	82.0±16.9	198.0±65.4	30.7±8.5	310.7±84.9	28.2±4.1	61.6±7.4	10.6±3.2
Control	81±6.5	107.0±8.2	10.3±2.2	198.3±8.6	48.5±5.2	53.8±2.3	5.2±1.1
Lh14	200.7±41.0	167.0±58.2	4.0±3.1	371.7±55.9	57.0±11.9	43.0±10.7	0.9±0.7
Control	81±6.5	107.0±8.2	10.3±2.2	198.3±8.6	48.5±5.2	53.8±2.3	5.2±1.1
LhSw	213.0±39.0	165.0±49.4	6.7±3.8	384.7±91.7	53.8±2.7	41.8±2.4	1.5±0.7
Control	44.3±2.7	201.0±17.0	41.7±8.4	287.0±26.2	34.0±17.9	70.1±0.6	14.3±1.9
LvPhil	88.3±23.8	122.3±8.6	28.0±3.6	238.7±18.6	28.3±8.7	52.0±6.3	11.9±2.0
Control	44.3±2.7	201.0±17.0	41.7±8.4	287.0±26.2	34.0±17.9	70.1±0.6	14.3±1.9
LvHaw	77.7±16.8	225.7±22.0	24.0±6.6	327.3±33.2	20.0±3.4	69.0±0.5	7.8±2.8
Control	166.3±36.3	239.7±31.7	39.0±25.0	445.0±93.0	44.4±7.2	55.4±3.7	7.4±3.4
LgCam	370.0±37.6	169.0±18.5	9.0±4.7	548.0±52.2	67.0±0.7	30.8±1.4	1.8±1.1
Control	166.3±36.3	239.7±31.7	39.0±25.0	445.0±93.0	44.4±7.2	55.4±3.7	7.4±3.4
LgSA	221.0±58.2	117.3±12.8	4.0±2.0	342.3±64.2	67.0±5.8	35.8±5.1	1.1±0.6
Control	132.0±15.9	63.3±14.3	13.7±2.4	209.0±14.2	46.6±13.5	30.2±6.1	6.4±0.8
LcNet	153.3±9.6	118.0±38.5	27.3±2.0	298.7±47.7	47.5±4.2	37.6±6.1	9.5±1.3
Control	166.3±36.3	239.7±31.7	39.0±25.0	445.0±93.0	44.4±7.2	55.4±3.7	7.4±3.4
LcAtl	179.7±11.1	117.3±71.5	3.7±2.7	300.7±63.7	81.3±2.2	33.2±13.9	1.0±0.6
Control	66.3±7.9	100.3±51.5	8.7±2.2	175.3±59.5	57.2±5.8	51.2±9.1	5.3±0.8
GxUg	98.3±13.5	38.3±10.2	6.7±2.9	143.3±21.5	73.5±6.0	26.4±6.2	4.4±1.5
Control	160.7±39.1	54.7±5.6	10.7±2.3	226.0±40.5	58.2±14.0	25.2±3.6	5.2±1.8
GxHaw	137.7±19.9	74.0±9.0	8.7±2.6	220.3±25.0	62.4±3.1	33.6±2.0	4.2±1.5

B.

	CELL COUNTS				PERCENT of TOTAL CELLS		
48h post-infection	**Plasmatocytes±SEM**	**Podocytes±SEM**	**Lamellocytes±SEM**	**Total Cells±SEM**	**Plasmatocytes±SEM**	**Podocytes±SEM**	**Lamellocytes±SEM**
Control	57.7±10.8	28.7±3.2	5.7±0.3	92.0±13.2	61.9±3.7	31.7±3.5	6.3±0.6
Lb17	80.3±19.4	54.0±2.3	15.3±0.9	149.7±19.2	51.9±7.0	37.5±5.7	10.6±1.4
Control	112.3±22.4	77.7±25.3	24.7±9.3	214.7±31.4	53.5±10.9	35.3±7.7	11.2±3.3
LbG486	99.0±20.6	70.3±38.5	34.3±8.6	203.7±5.6	52.6±6.9	29.3±8.4	18.1±3.9
Control	132.0±30.4	91.0±18.0	24.0±7.5	247.0±45.3	52.8±5.9	37.9±5.7	9.2±1.5
Lh14	124.3±10.4	63.3±7.1	8.0±2.5	195.7±5.6	63.4±3.9	32.4±3.7	4.2±1.4
Control	255.7±16.0	255.0±16.4	111.7±45.0	622.3±54.9	41.8±4.8	41.2±2.2	17.0±5.3
LhSw	203.7±21.7	214.0±26.2	26.3±2.3	444.0±33.2	45.9±3.4	48.0±3.8	6.1±1.0
Control	71.3±11.9	28.7±3.2	7.7±1.7	107.7±16.5	66.0±0.8	27.0±1.3	7.0±0.6
LvPhil	97.7±12.3	46.3±13.3	20.3±8.4	149.3±11.8	56.6±8.2	30.2±7.4	13.1±4.7
Control	203.0±41.0	255.7±78.9	166.0±14.6	624.7±128.3	32.8±1.9	38.5±6.0	28.7±5.3
LvHaw	215.3±20.0	227.3±27.2	90.3±2.0	495.0±33.5	35.9±2.1	45.7±2.7	18.5±1.7
Control	112.3±22.4	77.7±25.3	24.7±9.3	214.7±31.4	53.5±10.9	35.3±7.7	11.2±3.3
LgCam	82.7±4.8	91.7±27.0	4.7±0.9	194.0±35.0	51.8±6.5	45.7±6.3	2.5±0.6
Control	112.3±22.4	77.7±25.3	24.7±9.3	214.7±31.4	53.5±10.9	35.3±7.7	11.2±3.3
LgSA	177.3±14.8	98.0±20.7	8.0±5.1	321.3±20.2	67.4±6.8	30.2±5.3	2.4±1.5
Control	132.0±30.4	91.0±18.0	24.0±7.5	247.0±45.3	52.8±5.9	38.0±5.7	9.2±1.5
LcNet	94.3±16.8	89.7±16.8	48.7±4.3	232.7±31.4	40.0±2.3	38.1±3.1	21.9±3.9
Control	323.3±28.5	236.3±57.1	47.3±11.2	607.3±39.6	54.1±7.4	38.2±6.9	7.6±1.5
LcAtl	354.3±47.6	155.3±4.8	18.0±7.0	527.7±42.0	66.6±4.2	29.9±3.1	3.5±1.5
Control	92.3±16.6	76.3±10.4	41.7±11.6	210.3±5.6	43.8±7.7	36.6±5.8	19.7±5.1
GxUg	165.3±19.5	45.7±23.9	7.7±2.0	218.7±22.0	76.9±10.5	19.7±9.8	3.4±0.7
Control	122.0±11.0	156.0±55.2	38.0±14.6	316.0±64.7	42.2±10.0	46.6±8.4	11.2±1.9
GxHaw	113.0±8.1	137.0±29.5	12.0±6.1	262.0±39.5	44.5±4.5	51.1±4.9	4.4±1.8

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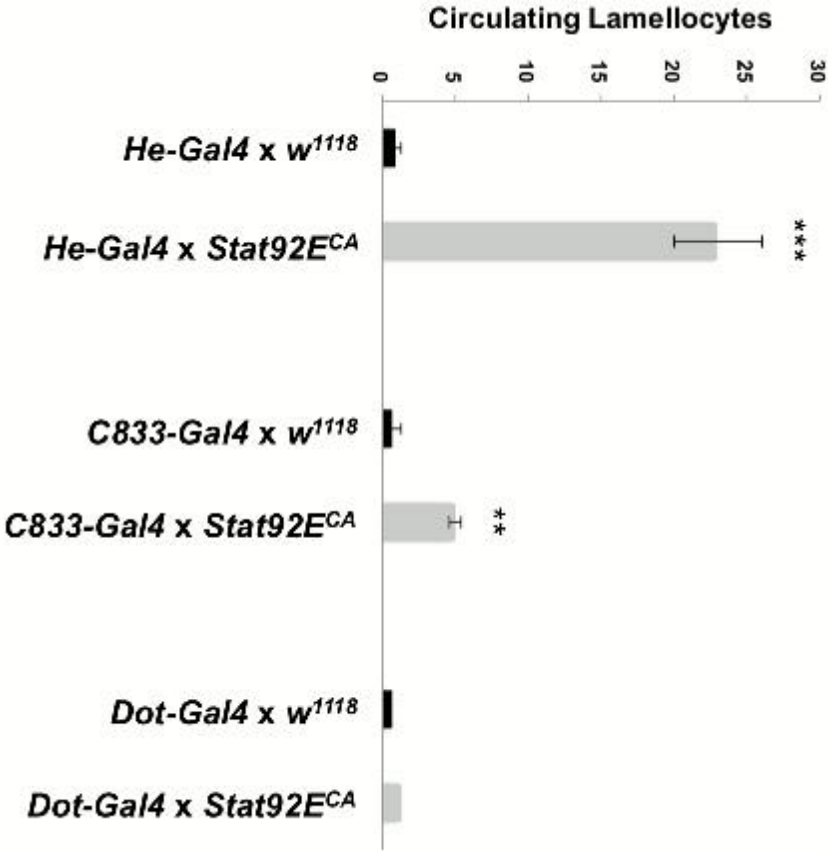
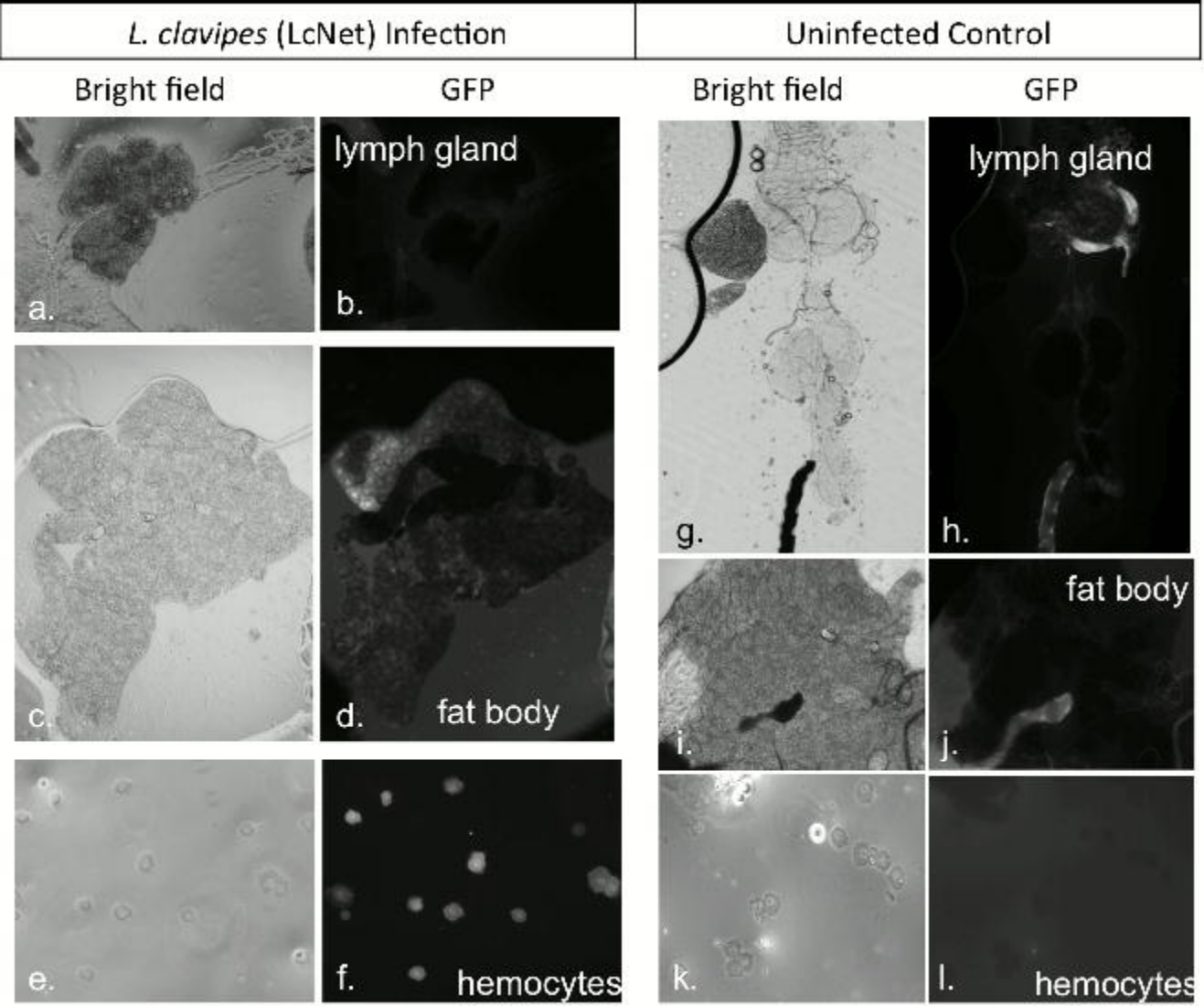
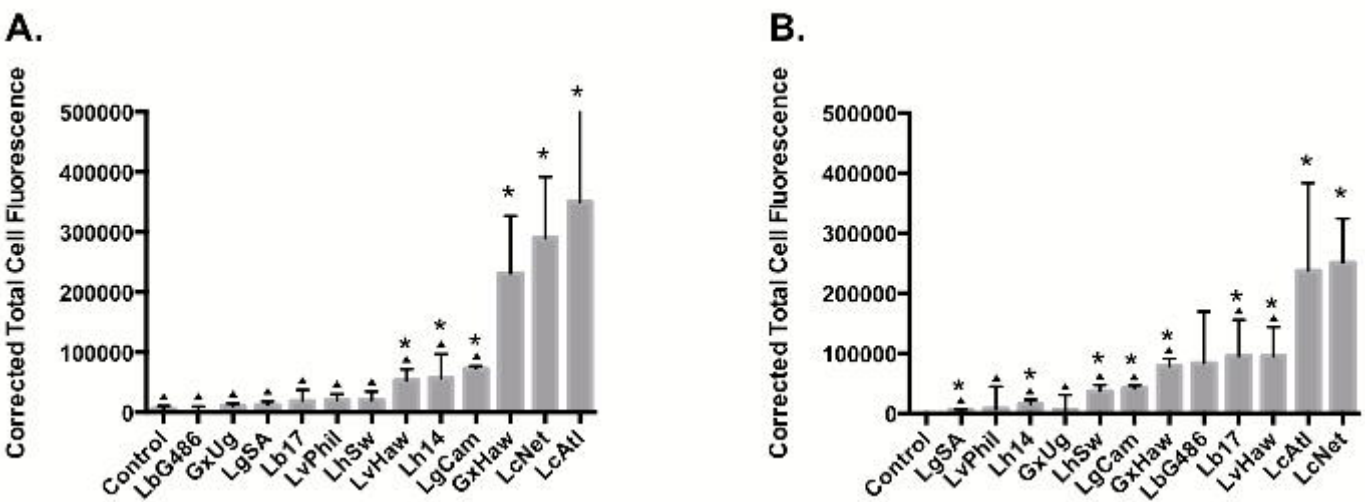
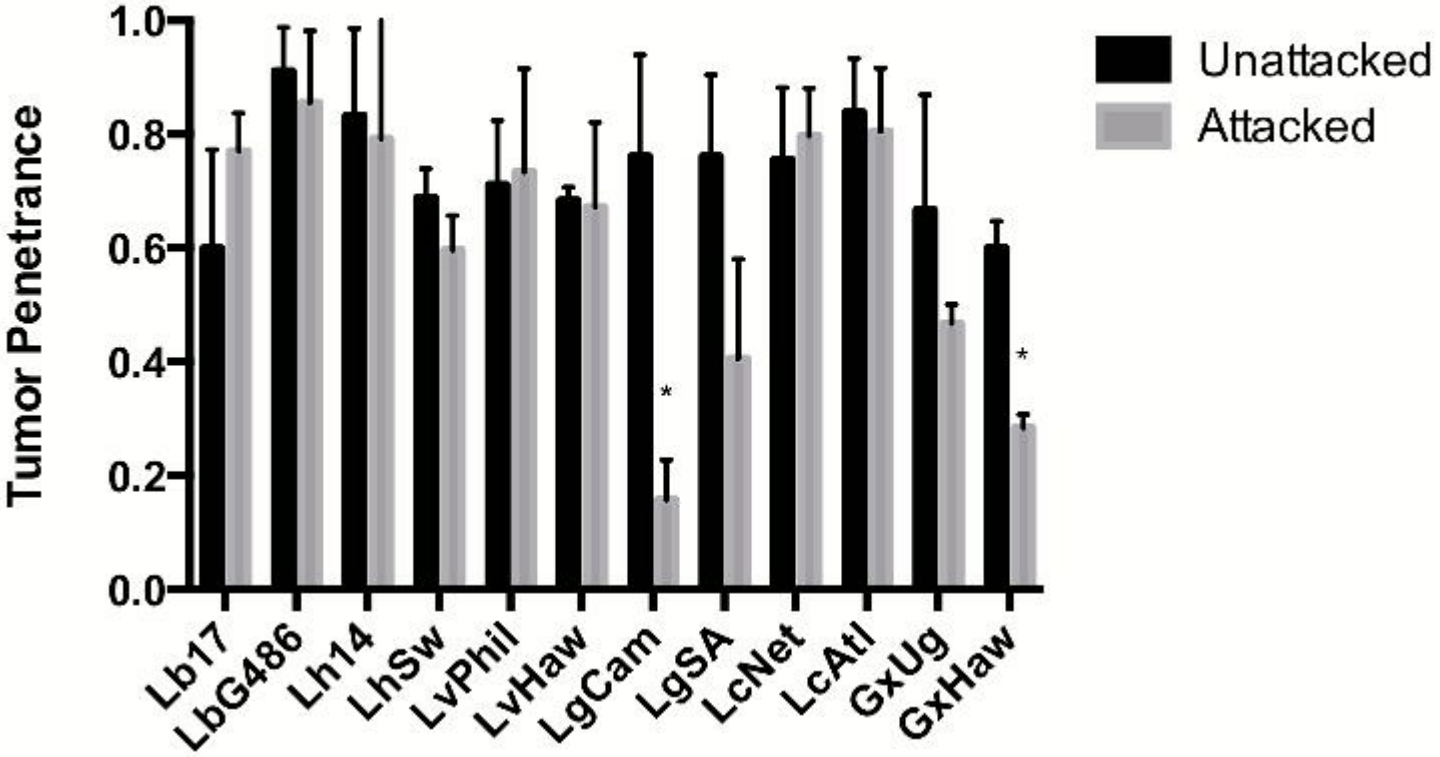
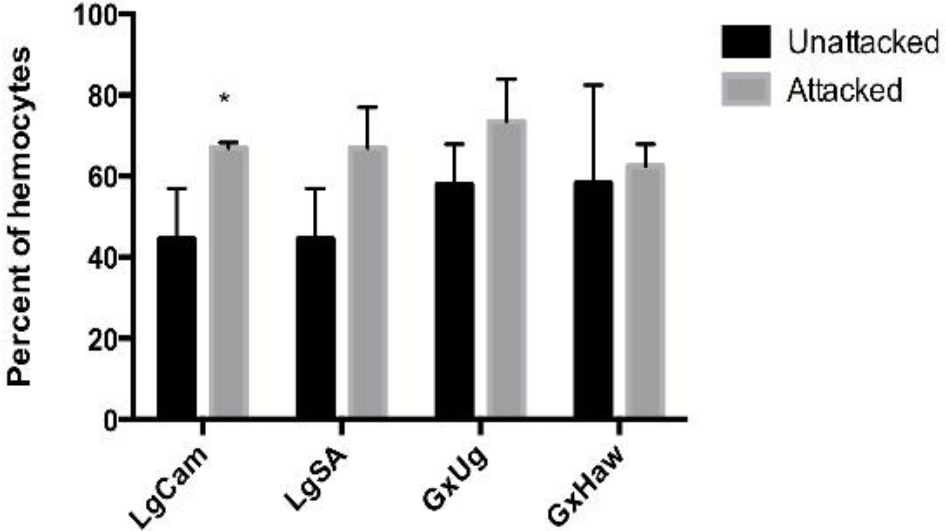

Circulating Lamellocytes
30
25
20
15
10
5
0
He-Gal4 x w1118
He-Gal4 x Stat92ECA
***
C833-Gal4 x w1118
C833-Gal4 x Stat92ECA
**
Dot-Gal4 x w1118
Dot-Gal4 x Stat92ECA


L. clavipes (LcNet) Infection
Uninfected Control
Bright field
GFP
Bright field
GFP
lymph gland
a.
b.
c.
d.
fat body
e.
f.
hemocytes
lymph gland
g.
h.
i.
fat body
j.
k.
l.
hemocytes

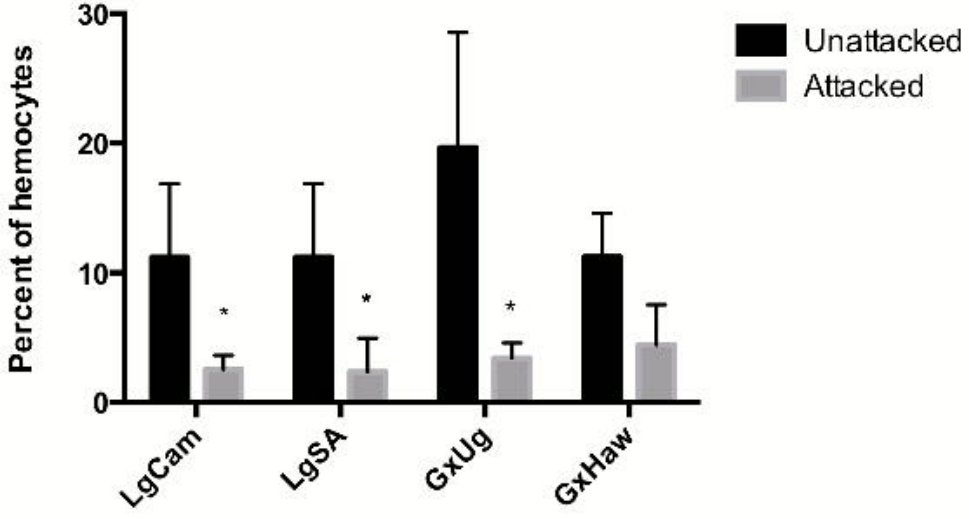

A.
Corrected Total Cell Fluorescence
500000
400000
300000
200000
100000
0
Control
LbG486
GxUg
LgSA
Lb17
LvPhil
LhSw
LvHaw
Lh14
LgCam
GxHaw
LcNet
LcAtl
B.
Corrected Total Cell Fluorescence
500000
400000
300000
200000
100000
0
Control
LgSA
LvPhil
Lh14
GxUg
LhSw
LgCam
GxHaw
LbG486
Lb17
LvHaw
LcAtl
LcNet

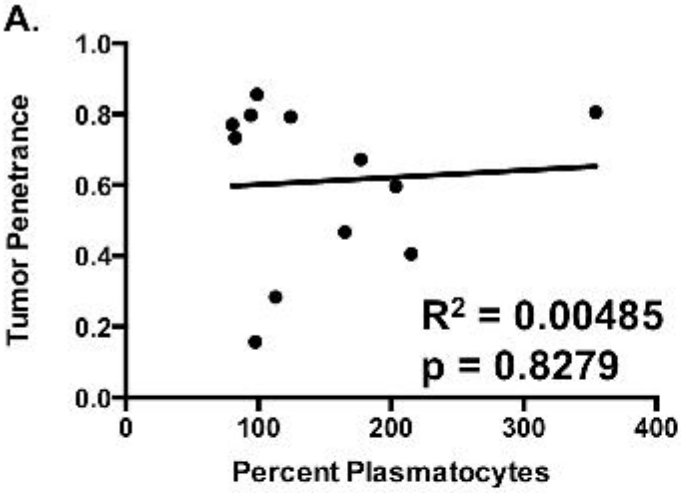

Tumor Penetrance
1.0
0.8
0.6
0.4
0.2
0.0
Unattacked
Attacked
*
*
Lb17
LbG486
Lh14
LhSw
LvPhil
LvHaw
LgCam
LgSA
LcNet
LcAtl
GxUg
GxHaw

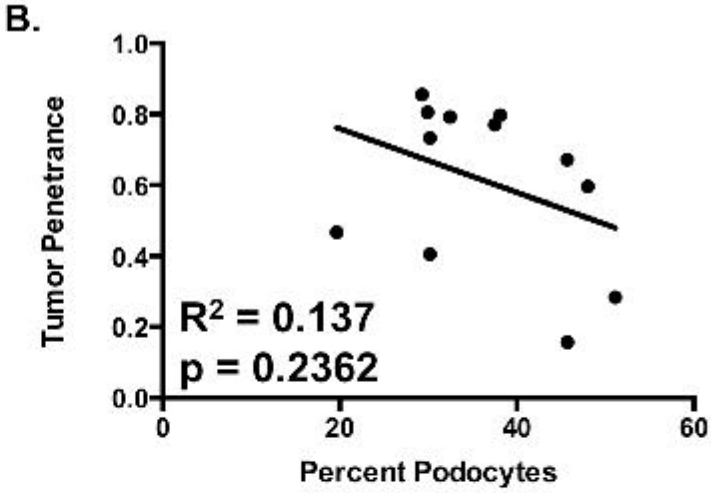
A.


Percent of hemocytes
100
80
60
40
20
0
*
LgCam
LgSA
GxUg
GxHaw
Unattacked
Attacked

B.


Percent of hemocytes
30
20
10
0
*
*
*
LgCam
LgSA
GxUg
GxHaw
Unattacked
Attacked


A.
Tumor Penetrance
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
Percent Plasmatocytes
R² = 0.00485
p = 0.8279


B.
Tumor Penetrance
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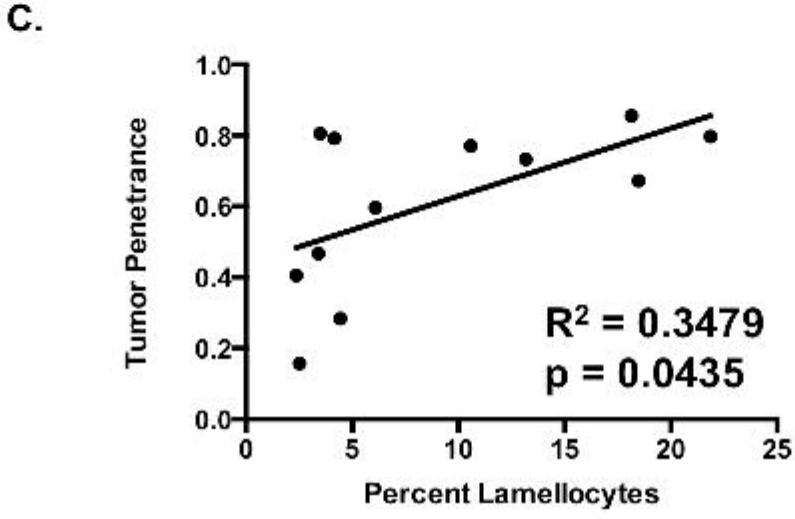
0.8
0.6
0.4
0.2
0.0
0
20
40
60
Percent Podocytes
R² = 0.137
p = 0.2362


C.
Tumor Penetrance
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0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
Percent Lamellocytes
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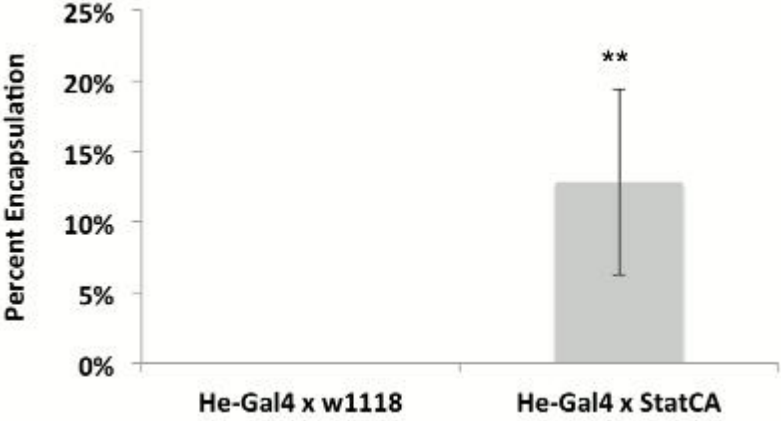
p = 0.0435


25%
20%
15%
10%
5%
0%
Percent Encapsulation
**
He-Gal4 x w1118
He-Gal4 x StatCA